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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C08B 37/16, A61K 47/40

A1

(11) International Publication Number: **WO 99/45032**

(43) International Publication Date: 10 September 1999 (10.09.99)

(21) International Application Number: PCT/FI99/00167

(22) International Filing Date: 4 March 1999 (04.03.99)

(30) Priority Data:
980489 4 March 1998 (04.03.98) FI

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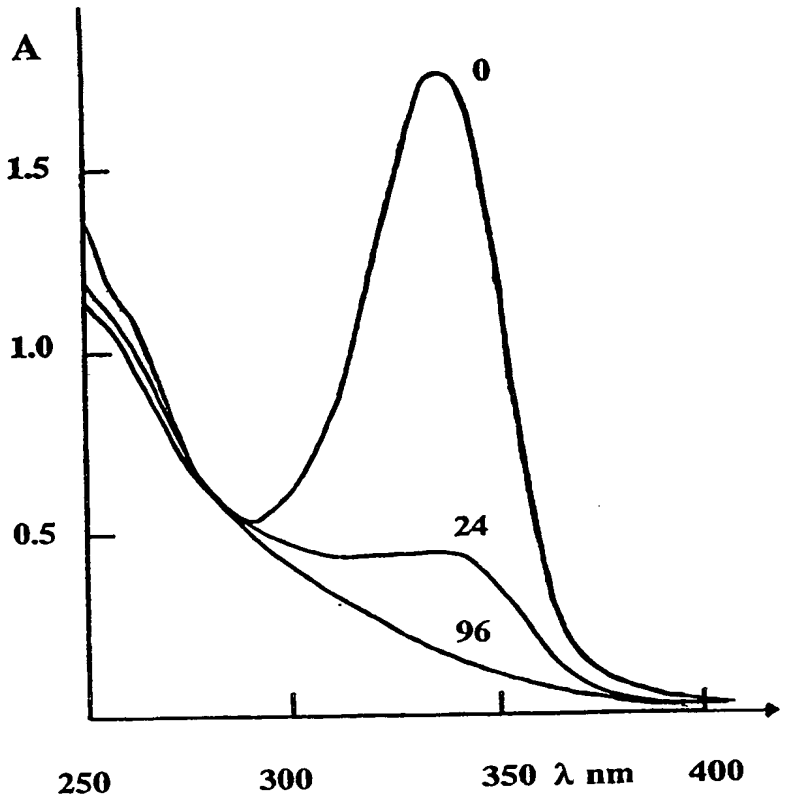
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: NOVEL DERIVATIVES OF CYCLODEXTRINS

(57) Abstract

The present invention is directed to novel aminooxy–cyclodextrin derivatives of the formula (1): CD – (X – Y – ONH_2)$_n$, wherein CD is a mono– or polydeoxy α–, β–, or γ–cyclodextrin, carrying in its 6–, 3– and/or 2–position a group containing the aminooxy group, and optionally carrying substituents different from (X–Y–ONH_2), Y is a linker group between the aminooxy group and the mono– or polydeoxy–CD group, X is a functional group or an atom necessary to connect the linker Y and the deoxy CD group, or Y is a direct bond when X is a direct bond, and n is ≥ 1, but ≤ 24, 21 and 18 for α–, β– or γ–cyclodextrin, respectively, the protected aminooxy derivatives thereof, as well the methods for their preparation and use.



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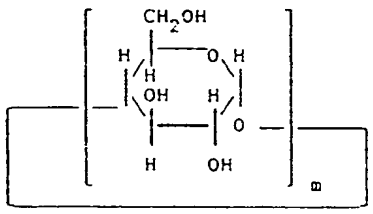
NOVEL DERIVATIVES OF CYCLODEXTRINS

FIELD OF THE INVENTION

This invention relates to the design and synthesis of earlier unknown chemical derivatives of cyclodextrins. The novel compounds exert a number of useful properties which make them applicable as complexants, solubilizers, carbonyl reagents, catalysts, or starting materials for the synthesis of products to be employed in pharmaceuticals, cosmetics, agriculture or in scientific laboratories.

BACKGROUND OF THE INVENTION

α-, β- and γ-Cyclodextrins (α-CD, β-CD and γ-CD) are cyclic oligosacharides consisting of 6, 7 or 8 glucopyranose units, respectively, which are joined together by α(1-4) linkages:



m=6 α-CD
m=7 β-CD
m=8 γ-CD

Cyclodextrins (termed hosts) can imbibe certain molecules or parts thereof (termed guests) into their center cavities. The noncovalent reversible adducts or inclusion complexes formed between the host and the guest can drastically change the properties of the parent guest molecules in diverse ways, such as to increase solubility, decrease volatility, protect from chemical or light-catalyzed reactions, change the location of absorption of complexed drugs in the intestine, etc.

Parent CDs can be covalently modified with a number of reagents to form chemical derivatives. The derivatives can

normally bind similar guest compounds as do the parent CDs, but the properties of the complexes can be changed. A description on the syntheses of CD derivatives and the properties of inclusion complexes of both parent and modified cyclodextrins can be found for instance in *Croft, A.P. & Bartsch, R.A. "Synthesis of Chemically Modified Cyclodextrins", Tetrahedron, 1983, V. 39, No 9, P. 1417-14-71 Szejtli, J. "Cyclodextrin Technology", Kluwer Academic Publishers, Dordrecht*, 1988, pp. 1-450.

Some derivatives of β-CD have a higher solubility than do the parent compound and hence they are often preferable complexants and solubilizers. The potential of the chemical derivatives of β-CD is amplified by its low price as a starting material in comparison to α- and γ-CDs. In contrast to β-CD, the more expensive α- and γ-CDs are readily water soluble and can be used without chemical derivatization for certain purposes. This is illustrated by a number of reports on their complexes with various guest compounds such as steroid hormones, cholesterol or its derivatives and with some drugs. Appropriately alkylated or hydroxyalkylated γ-CDs are also good complexants since their inclusion complexes do not precipitate even at high concentration, as stated in EP 06792.

A large number of papers deals with the syntheses of CD chemical derivatives and their application for divergent purposes (see e.g. *Szejtli, J. "Cyclodextrin Technology" 1988*) clearly showing the importance of the CD derivatives.

SUMMARY OF THE INVENTION

The present invention describes novel CD derivatives carrying specific functions containing an aminooxy (H_2NO-) group covalently connected to a glucopyranose unit of CD. These derivatives have significantly different properties from the CD derivatives known in the prior art and thus

they enlargen the area of application of CDs. The present invention also describes the preparation and use of the said novel CD derivatives as such or complexed with guest molecules or further chemically modified.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is related to an earlier unknown type of α-, β- or γ-CD chemical derivatives containing the aminooxy (H_2NO-) functional group attached to the CD core and having the general formula 1:

$$CD - (X - Y - ONH_2)_n \qquad \text{formula 1}$$

wherein CD is mono- or polydeoxy α-, β- or γ-CD, carrying in its 6-, 3- and/or 2-position the aminooxy function containing group, and wherein Y is a linker arm connected to deoxy-CD by means of X, which is a direct bond, or a functional group or an atom necessary to connect the linker Y and the deoxy-CD, whereby Y is a direct bond when X is a direct bond. The integer n is equal to or larger than one and cannot be more then 18, 21 and 24 for α-, β- and γ-CD, respectively.

The invention also relates to the compounds of the formula 1, wherein the aminooxy group is in protected form, especially in the form of the 1-ethoxy-ethylideneaminooxy group, $-O-N=C(CH_3)OC_2H_5$, or as the acetone oxime group, $-O-N=C(CH_3)_2$.

The aminooxy-CDs of formula 1 preferably carry one or several H_2NO-groups attached to 6-hydroxy groups (see examples I-IV). By utilizing the different reactivities of primary and secondary hydroxyl groups (primary hydroxyls more reactive than secondary), and if necessary, suitably protected hydroxyl groups, one can discriminate between the reaction at the "top" (primary) hydroxyls and at the

"bottom" (secondary) hydroxyls of the CD molecules (see *Croft et. al. supra*). These latter types may be important for synthetizing artificial receptors, carriers and catalysts based on the CD-core.

In the compound of the formula 1, CD is a mono- or polydeoxy α-, β- or γ-cyclodextrin. In these compounds, one or more hydroxy groups in the positions 6, 3 and/or 2 of CD are replaced with a (X-Y-ONH_2) fragment, and specifically, together with primary hydroxy groups, one or more secondary hydroxy group can also be substituted with a (X-Y-ONH_2) fragment. The compound according to the invention carrying aminooxy groups can optionally carry further substituents. In the aminooxy-CD, one or more hydroxy groups in the 6-, 3- and/or 2-position may be also substituted e.g. into H_2N-, HS-, -COOH, alkoxy, such as C_1-C_6-alkoxy, aryloxy, aryl being preferably phenyl, benzyl or tolyl, or acyloxy groups, acyl being preferably derived from C_1-C_6-carboxylic or benzoic acid. Alkyl-, aryl- and acyloxy may carry additional functional groups in a side chain or aromatic ring.

Y is a "linker arm, or linking group" and serves as a bridge between the aminooxy (H_2NO-) group and the deoxy-CD moiety. Usually Y is alkylene, alkenylene with one or more double bounds which may be either isolated or conjugated, alkynylene with one or more triple bonds which may be either isolated or conjugated, or arylene or arylalkylene fragments where aryl may be substituted or not substituted, such as phenylene. The alkylene, alkenylene and alkynylene fragments may be linear or branched and preferably contain 2-12 C-atoms in the chain. One or more of the chain members (methylene groups) may be replaced by -NH-, -O-, -S-, -S-S-, -C(O)NH, -C(O)O-, -OP(O)(OH)O-, -S(O)-, SO_2-, -CHR-, where R is preferably alkyl, aryl, -OR', $-NH_2$, -NHR', $-NR'_2$, -OH, -COOH, or $-ONH_2$ groups and where

R′ is alkyl, aryl, or acyl. As R and R′, aryl is preferably phenyl, aryl lower alkyl, such as benzyl or tolyl.

X is preferably -O-, -S-, -NH-, -NR"-, -OCO-, -NH-O-, =NO-, -NHC(O)-, -OP(O)(OH), -R"C=NO-, where R" is alkyl.

R, R′ and R" when having the meaning of alkyl, are preferably linear or branched C_1 - C_6-alkyl, in the meaning of acyl they are preferably derived from linear or branched C_1-C_6-carboxylic acids or benzoic acid.

In the preferred compounds of formula 1, Y is alkylene or alkenylene of 2-12, preferably 2-6 C-atoms, wherein one or more of the chain members may be replaced by -NH-, =N-O-, -O-, -S-, -C(O)NH-, -C(O)O-, or $-CHR_1-$ wherein R_1 is methyl, ethyl or propyl and X is -O-,-S-,-NH-,-OC(O)- or -NH-O-.

The compounds of the formula 1 are week bases (usually the pK of the H_2NO-group is between 4.0 - 6.0) and their solubility is different from the parent CD molecules. As indicated by the pK values, a unique possibility exists to regulate the ionic form of the compounds of formula 1 by solvent acidity near the physiological pH-region. That means that a low pH favours complexation of ionic guest molecules, while high pH-values favour the contribution of non-ionic interactions between host and guest. With related compounds containing alkylamino functions, protonation-deprotonation takes place only at around pH 10 (*Boger,J. et al. Helv.Chim.Acta,1978, V. 61, P. 2190-2218)*.

The compounds of formula 1 are carbonyl reagents like other O-substituted hydroxylamines. They react rapidly and quantitatively with various aldehydes and ketones forming oximes which have high stability in water solution at a broad range of pHs. These properties of aminooxy-CDs enable the synthesis of a multitude of CD derivatives in addition to those of the formula 1; for example, immobilization of

CDs on solid supports and subsequent use in the chromatography of various important compounds such as stereoisomers of pharmaceuticals. In addition, oligo- and polymeric materials are readily obtained in a single-step by allowing dialdehydes or diketones to react with di- or polysubstituted aminooxy-CDs in aqueous solution. Such oligo- or polymeric materials are advantageously used as semipermeable or stereoselective membranes, as prolonged-release supports for drugs, sanitary, cosmetics or agricultural materials. Further, the chemical reactivity properties of the aminooxy functions enable one to stabilize CD-complexes of certain physiologically active, highly reactive, aldehydes and ketones - for instance, steroids, prostaglandins and vitamins - by anchoring these into CDs via the oxime bond in addition to the stabilization involved in the host-guest interaction. Since the stabilization effect is cumulative (not additive), the protection conferred by molecular complexation can be drastically increased.

Inclusion complexes in general may be additionally stabilized by means of oxime formation with a suitable aldehyde or ketone. In this case the inclusion complex is first formed which is then reacted with the aldehyde or ketone to form the inclusion complex oxime. Thus the existence of steric hindrance at the cavity entrance may prevent complex from dissociation.

While the oxime bond is stable in water solutions, especially at extreme pH values, it may slowly decompose. This property can be utilized for the slow release of aminooxy-CD bound drugs in the stomach and intestine.

Since aminooxy-CDs are carbonyl reagents, they may inhibit certain crucial enzymes in the metabolism of cells, such as PLP-, pyruvate-, or ketobutyrate-dependent enzymes. The inhibitory potency will depend on the affinity of the coenzyme to protein.

The existence of aminooxy group(s) bound to a CD molecule means that such compounds, like other O-substituted hydroxylamines, are capable of reacting directly with cytidine and adenosine. This was confirmed by the reaction of aminooxy-CDs of formula 1 with 4-thiouracil, 6-mercaptopurine riboside or their derivatives and even cytidines themself (see examples XIII and XIV). Hence, the compounds of formula 1 can be useful for the modification of nucleotides, nucleosides, bases, nucleoside coenzymes and nucleic acids, such as for the formation of nucleotide and nucleoside pyrimidine and purine derivatives of aminooxy CD, wherein the pyrimidine and purine preferably are cytosine or adenine as such, or in the form of their corresponding derivatives.

At neutral and slightly acidic pH, the aminooxy groups of compound of formula 1 are not protonated. The nonprotonated aminooxy groups are strong nucleophiles capable of reacting with an activated carboxyl group (esters, activated esters, mixed anhydrides, anhydrides, etc.) even in water solutions forming stable hydroxamic acids. These can have new useful properties such as the ability to complex certain metal ions. Combined metal ion and CD complexation functions of the aminooxy-CD derivatives may be used for recovering of metal ions from solutions.

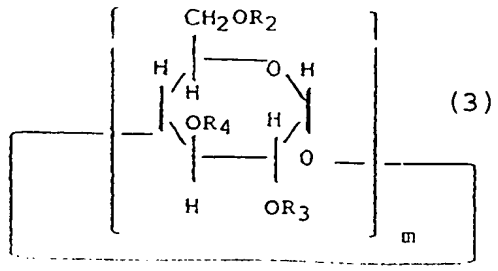
A comparison of the compounds of the present invention with amino group containing CDs *(Boger, J. et al. Helv.Chim.Acta, 1978, V. 61, P. 2190-2218)* demonstrates various advantages for the aminooxy-CDs. The basic disadvantages of the alkylamino-CDs are the high pKs necessitating alkaline reaction conditions during the derivatization reactions and the low stability of the Schiff-base bond between the amino and aldehyde or keto groups in aqueous solutions.

The high nucleophilicity of the aminooxy (H_2NO-) group,

its easy introduction into different sites of the CD molecules with different spacer arms make the aminooxy-CDs and their derivatives promising for the construction of catalytically active CDs.

The compounds of formula 1 can be prepared in different ways, and the present invention is also directed to the processes for the preparation of the novel compounds of the formula 1. Such processes are:

a) alkylation of a corresponding CD derivative with an aminooxy-protected, reactively substituted aminooxy derivative, for example with a compound of the formula 3':



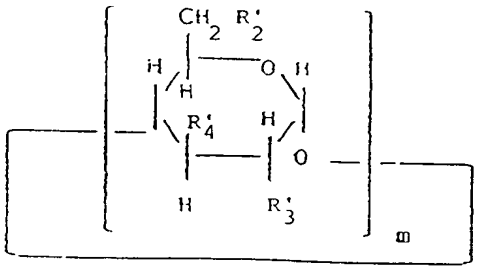
(3)

$$Z - Y - ON=C(CH_3)W \qquad (3')$$

wherein R_2, R_3 and R_4 are independently hydrogen, or a substituent (see *Croft et. al. supra*) having no reactive functional group, being typically alkyl, such as C_1-C_6-alkyl, or aryl, such as phenyl, benzyl or tolyl, whereby at least one of the positions 6, 3 and/or 2 contains a hydroxy group, preferably the 6-hydroxy group, W means OC_2H_5 or CH_3, m and Y are as defined above and Z is a reactive group, such as Cl, Br, I, tosyl or mesyl, and optionally removing the protecting group. In this case a compound of the formula 1 is obtained, wherein X is O. In the above formula, when W is OC_2H_5, the compound (3) is protected in the form of the 1-ethoxy-ethylideneaminooxy derivative, and when W is CH_3, in the form of the acetone oxime derivative.

Suitable compounds (3') are e.g. 4-(ethoxyethylideneaminooxy)bromobutene-2, ethyl N-(ω-iodoalkyloxy)acetimidate, the sodium salt of 3-(ethoxyethylideneaminooxy)-2-bromo-bromopropionic acids etc. The compounds (3') are used in alkaline water solutions, using e.g. alkali or alkaline-earth metals, or hydrides, hydroxides, oxides, carbonates, hydrocarbonates thereof; or quaternary ammonium salts, mono-, di- and trialkylamines carrying lower (C_1-C_4) linear or branched alkyl groups being the same or different in alkaline water-organic mixtures (the organic solvent being e.g. a lower (1-4C) alcohol, dioxane, tetrahydrofuran, glyme, cellosolve, dimethylsulfoxide, dimethylformamide) or in liquid ammonia at temperatures from elevated (about 100°C or higher) to ambient temperature. The substitution degree depends on the reaction conditions and the products can have either only few primary hydroxyls being substituted or also secondary hydroxy groups may be involved in the reaction.

b) Alkylating an activated CD-derivative, such as a tosylate, mesylate, halogen derivative, epoxide, activated ester, with an aminooxy-protected, functionally substituted hydroxylamine



$$HX - Y - ON=C(CH_3)W \qquad (4')$$

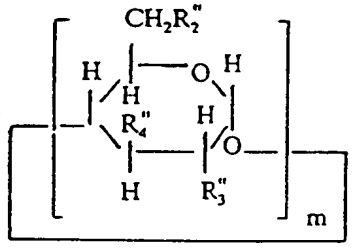
wherein R'_2, R'_3 and R'_4 are hydroxy, or an activated group such as tosyl, mesyl, halogen, ester, epoxide, and X, Y and W are as defined above, and thereafter optionally removing the protecting group. In this reaction, aminooxy CD derivatives are obtained, wherein X is not only O, but also e.g. sulfur or an imino group.

Suitable compounds of the formula (4) are e.g. ethyl N-(ω-mercaptoalkyloxy)-acetimidate, ethyl N-(ω-aminoalkyloxy)acetimidate or ethyl N-hydroxyacetimidate itself.

The activated CD-derivative can also be e.g. a mono- or poly-N-hydroxysuccinimide activated CD-derivative having a -COOH group, which is reacted with the compound of formula 4, where X is a HN-group.

According to an embodiment, one or more of the secondary hydroxy groups in the CD derivative may be unsubstituted or substituted with groups other than activating tosyl, mesyl or halogen, such as with those described above.

c) Modifying a functionally-substituted CD derivative having of the formula (5)



(5)

wherein at least one of the groups R''$_2$, R''$_3$, and R''$_4$ mean thiol-, amino-, karboxy- etc. group possibly linked directly to deoxy-CD-ring, or mean alkylenoxy- or acyloxy groups, which contain at least one thiol-, amino-, karboxy-, etc. group, or their derivative, and the remaining funtional groups are hydroxyl groups or they have the meaning described in claim 7 for the substituents, and exist, if necessary, in a protected form, typical example being unsubstituted alkoxy, aryloxy, or acyloxy, modified with an appropriate aminooxy protected substituted hydroxylamine according to formula (3'), after which the protecting group(s) are removed, or

d) With modifying such CD-derivative, having one or more keto or aldehyde function at 2-, 3-, and/or 6-position, optionally joined with the above-described linkers, according to bis-aminooxyalkanes of formula (5')

$$H_2NO(CH_2)_tONH_2 \qquad (5')$$

wherein t = 2-12 and wherein one of the methylene groups can be replaced with O or S atoms or -NH- or -S-S- functions.

The cyclodextrin starting materials of the described reactions are well-known from literature.

Selection of a proper protecting group for aminooxy function is crucial, to be succesful in preparing the compounds of formula 1. In the present invention ethyl-N-hydroxyacetimidate fragment or alternatively acetonoxime were employed. Derivatives protected in such a way are stable in a large area of different reaction conditions and the derivatives can be readily converted to corresponding O-substituted hydroxylamines by acid treatment. In the case of ethoxylidene protection the masking group can be removed within 10-60 min at the room temperature with a diluted strong acid, exemplified by hydrohalides, sulfuric phosphoric, nitrous, and paratoluenesulfonic acids. On the contrary, removal of acetonoxime protection demands by refluxing with 20% (w/v) hydrochloric acid.

The invention is described in the following by nonlimiting examples.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1a. Time course of the UV spectra (optical path length 1 mm) of a reaction mixture containing 1 mM 4-thiouracil in 100mM 4-aminooxy-2-butenyl-beta-cyclodextrin (I; see Example I.2) at pH 7.0. Incubation at 20°C is indicated as hours.

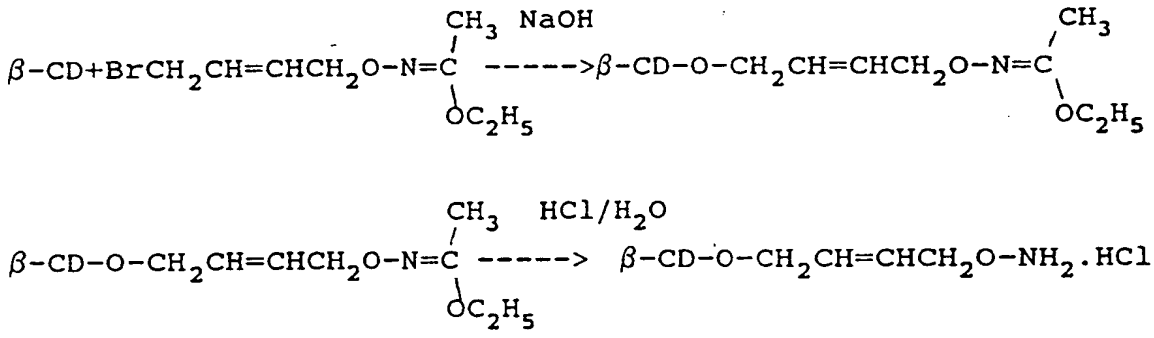
Figure 1b. Time course of the UV spectra (optical path length 1 mm) of a reaction mixture containing 1 mM 4-thiouracil and 100 mM 1-aminooxybutane at 20°C at pH 7.00. Incubation time (hours) is indicated.

Figure 2a. Time course of the UV spectra (optical path length 1 mm) of a reaction mixture containing 1 mM 6-mercaptopurineriboside with 100 mM 4-aminooxy-2-butenyl-beta-cyclodextrin (see Example I.2). Incubation time (hours) is indicated.

Figure 2b. Time course of the UV spectra (optical path length 1 mm) of a reaction mixture containing 1 mM 6-mercaptopurineriboside with 100 mM 1-aminooxybutane at pH 7.00. Incubation time (hours) is indicated.

Example I

I. 4-Aminooxy-2-butenyl-β-cyclodextrin hydrochloride (I).



β-CD-O-CH_2CH=CHCH_2O-NH_2.HCl

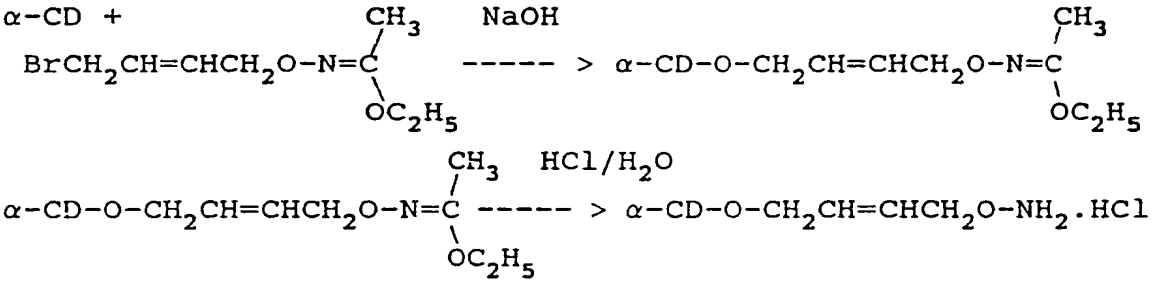
1. 3.1 g (14 mmoles) of 1-ethoxyethylideneaminooxy-4-bromo-butene-2 *(Khomutov A.R. and Khomutov R.M. (1986) Bioorgan.Khim., Russ., v.12, No.12, p.1662-1674)* was added to a solution of 2.26 g (2 mmoles) of β-cyclodextrin in a mixture of 18.7 ml water and 1.3 ml of 10 N NaOH and heated under nitrogen on a boiling water bath with intensive stirring until the pH turned neutral (usually 60-90 min). On cooling an oil separated. The product was washed with cold water and dissolved in 50 ml of i-PrOH and 4 ml of 5.0 N HCl was added. After a 30-minute incubation at 20°C, the liquid was decanted and the residual oil crystallized with absolute i-PrOH. The precipitation was filtered and washed with absolute i-PrOH and dried over P_2O_5/KOH in vacuo, resulting in 2.80 g (90% yield) of (I). The amount of aminooxy groups was determined *(Korpela T.K. and Makela M.J. (1981) Anal.Biochem. v.110, No.2, p.251-258)* and was 2.8 mmols/g, the low value indicating that only primary hydroxyl groups had reacted. NMR (Jeol-400, DMSO-d_6) :

2. 7.7 g (35 mmoles) of 1-ethoxyethylideneaminooxy-4--bromobutene-2 was added to a solution of 5.6 g (5 mmoles) of β-cyclodextrin in a mixture of 70 ml of water and 3.4 ml

of 10 N NaOH and mixed with a magnetic stirrer at 20°C. After two days, 10 ml of i-PrOH was added and stirring continued at 20°C until the pH turned neutral (usually about 5-8 days). The solution was evaporated to dryness in vacuo, the residual oil washed with cold water and dissolved in 50 ml of i-PrOH and 10 ml of 5.0 N HCl was added. After a 30 minute incubation at 20°C, the liquid was decanted and the residual oil crystallized with absolute i-PrOH. The precipitation was filtered and washed with absolute i-PrOH and dried over P_2O_5/KOH in vacuo, resulting in 3.56 g (50% yield) of (I). The amount of aminooxy groups determined as above was 2.26 mmols/g. NMR data identical to that in ex.I.1.

Example II

4-Aminooxy-2-butenyl-α-cyclodextrin hydrochloride (II).

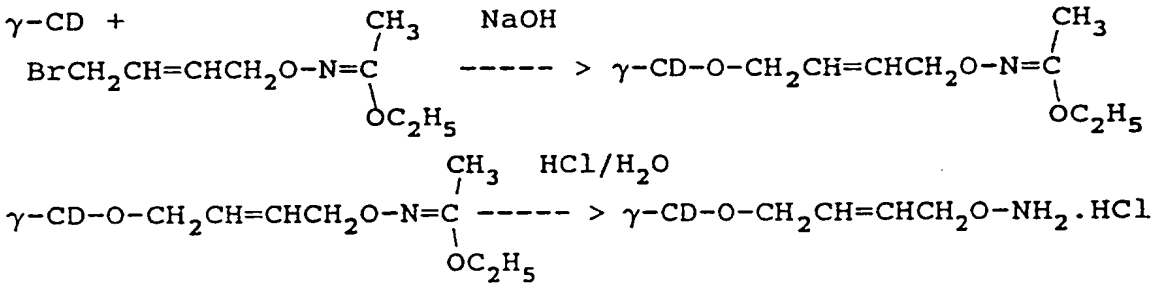


2.8 g (12 mmoles) of 1-ethoxyethylideneaminooxy-4-bromo-butene-2 was added to a solution of 1.96 g (2 mmoles) of α-cyclodextrin (Sigma) in a mixture of 18.9 ml of water and 1.1 ml of 10 N NaOH and heated under nitrogen on a boiling water bath with intensive stirring until pH turned neutral (usually 60-90 min). The oil which separated on cooling was washed with cold water and dissolved in 50 ml i-PrOH. To this solution 4 ml of 5.0 N HCl was added. After 30 min incubation at 37°C, the liquid was decanted and the residual oil crystallized with i-PrOH. The precipitation was filtered, washed with absolute i-PrOH and dried over

P_2O_5/KOH in vacuo resulting in 2.21 g (69% yield) of II. The amount of aminooxy groups determined as above was 3,25 mmoles/g. NMR (Jeol-400, DMSO-d_6) :

Example III

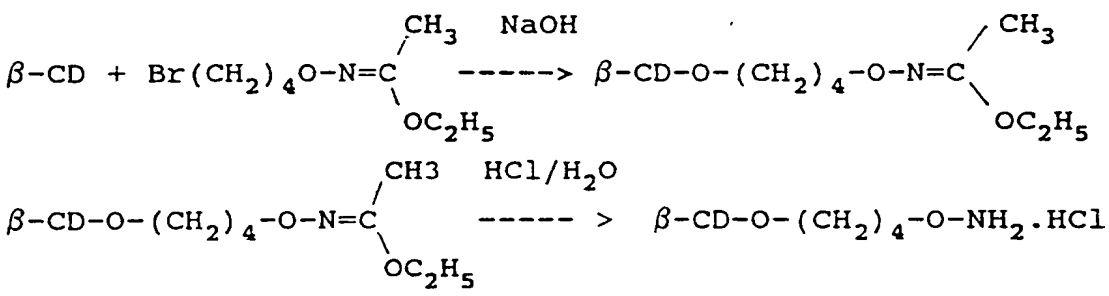
4-Aminooxy-2-butenyl-γ-cyclodextrin hydrochloride (III).



3.54 g (16 mmoles) of 1-ethoxyethylideneaminooxy-4-bromobutene-2 was added to a solution of 2.6 g (2 mmoles) of γ-cyclodextrin (Fluka) in a mixture of 18.5 ml of water and 1.5 ml of 10 N NaOH and heated under nitrogen on a boiling water bath with intensive stirring until the pH turned neutral (usually 60-90 min). The oil which separated on cooling was washed with cold water and dissolved in 50 ml of i-PrOH and 4 ml of 5.0 N HCl was added. After a 30-minute incubation at 20°C, the liquid was decanted and the residual oil crystallized with i-PrOH. The precipitation was filtered and washed with abs. i-PrOH and dried over P_2O_5/KOH in vacuo, resulting in 3.76 g (95% yield) of III. The amount of aminooxy groups determined as above was 2,86 mmoles/g. NMR (Jeol-400, DMSO- d_6):

Example IV

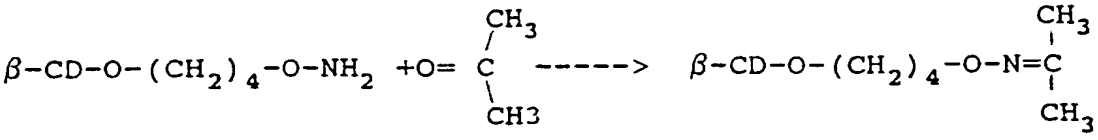
4-Aminooxybutyl-β-cyclodextrin hydrochloride (IV).



3.1 g (14 mmoles) of 1-ethoxyethylideneaminooxybutylbromide *(Nedospasov A.A. and Khomutov R.M. (1976) Izv. AN SSSR Ser. Khim. (in Russian) No.9, p.2113-2115)* was added to a solution of 2.26 g (2 mmoles) of β-cyclodextrin in a mixture of 18.7 ml water, 0.21 g (1.4 mmoles) of NaI and 1.3 ml of 10 N NaOH and heated under nitrogen on a boiling water bath with intensive stirring until the pH turned neutral (12-18 hrs). The oil separated on chilling was washed with cold water and dissolved in 50 ml of i-PrOH. To this solution 4 ml of 5,0 N HCl was added, after 30 min incubation at 20°C the liquid was decanted and the residual oil crystallized upon abs. i-PrOH treatment. The precipitation was filtered and washed with abs. i- PrOH and dried over P_2O_5/KOH in vacuum, that gave 1.66 g (58% yield) of IV. The amount of aminooxy groups determined as above was 2.20 mmoles/g. NMR (Jeol-400, DMSO-d_6) : 10.99 (m, H_2N-O-), 4.84 (m, C_1-H), 4.02 (m, H_2NO-CH_2-), 3.76-3.38 (mm, C_3-H, C_6-H, C_5-H, C_2-H, C_4-H), 1.62 (m, -CH_2-CH_2-). NMR (Jeol-400, D_2O) : 4.92 (m, C_1-H), 3.95 (m, H_2NO-CH_2-), 3.71-3.45 (mm, C_3-H, C_6-H, C_5-H, C_2-H, C_4-H), 1.59 (m, -CH_2-CH_2-).

Example V

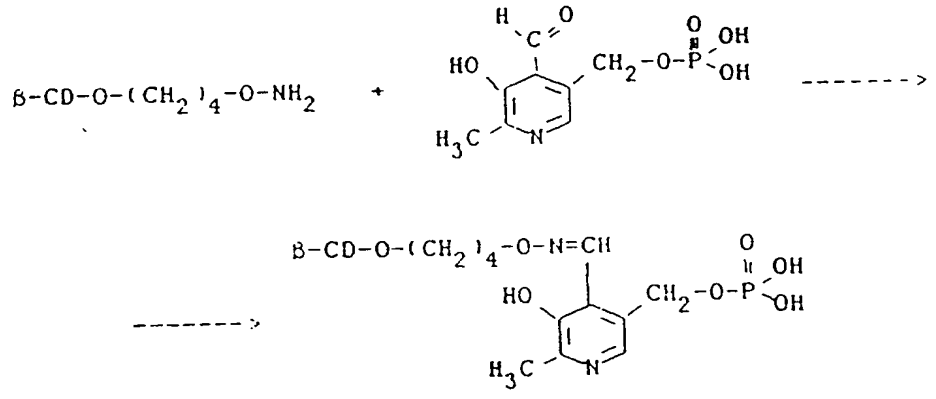
Acetonoxime of 4-Aminooxybutyl-β-cyclodextrin (V).



To a solution of 1.5 g of (IV) in 15 ml of H_2O-aceton mixture (1:1, V/V), diluted aqueous ammonia was added to a pH of 5-6. Then the reaction mixture was incubated for 2 h at 20°C. After evaporation to dryness, the residue was treated with water, the semi-solid product was separated and crystallized twice from water. The precipitate was filtered off, dried in vacuo over P_2O_5/KOH and 1.1 g (7 % yield) of (V) was obtained. NMR (Jeol-400, DMSO-d_6) : 4.83 (m, C_1-H), 3.92 (m, =NO-CH_2-), 3.75-3.22 (m, C_3-H, C_6-H, C_5-H, C_2-H, C_4-H), 1.77 (m, $(CH_3)_2C$=), 1.59 (m, -CH_2-CH_2-).

Example VI

Pyridoxal-5'-phosphate oxime of 4-aminooxybutyl-β-cyclo-dextrin (VI).

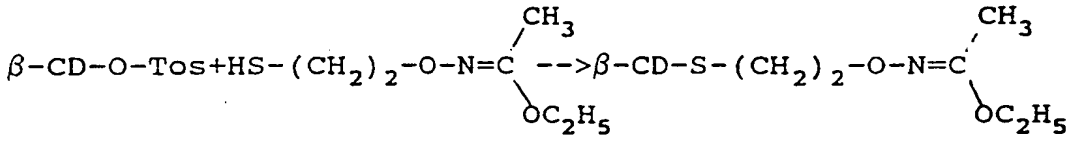


To a solution of 10.6 mg pyridoxal-5'-phosphate ("Merck") in 1.24 ml 0.1 N NaOD in D_2O, 20 mg of (IV) was added and

the reaction mixture was incubated for 2 hr. at 20°C. The compound (VI) was obtained with a yield being close to quantative. NMR (Jeol-400, D_2O) : 8.39 (m, H-C=N-O-), 7.70 (m, α-H), 4.85 (m, C_1-H), 4.74 (m, $-CH_2-O-P-$), 4.33 (m, H_2NO-CH_2-), 3.72-3.32 (m, C_3-H, C_6-H, C_5-H, C_2-H, C_4-H), 2.58 (m, α-CH_3), 1.58 (m, $-CH_2-CH_2-$).

Example VII

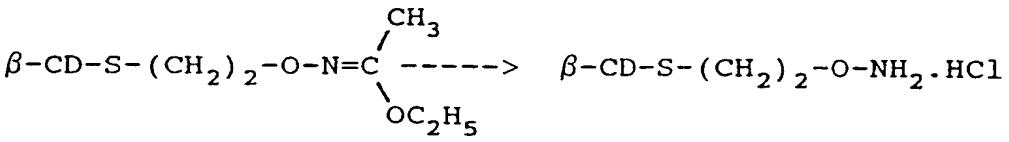
Mono-6-(2-ethoxyethylideneaminooxyethyl)thio-6-deoxy-β-cyclodextrin (VII).



To a solution of 0.74 g (4.5 mmoles) of 2-ethoxyethylideneamino-oxyethylmercaptane *(Khomutov A.R. and Khomutov R.M. (1986) Bioorg. Khim. (Russ.) v.12, No.12, p.1662-1674)* in 2.0 ml abs. MeOH was added 2.22 ml of 2 M MeONa/MeOH, after evaporation in vacuum to dryness the residue was dissolved in a mixture of 9.5 ml abs. DMSO and 0.5 ml MeOH and added to a solution of 1.95 g (1.5 mmoles) of mono-6-O-tosyl-β-cyclodextrin *(Matsui Y. and Okimoto A. (1978) Bull.Chem.Soc. (Japan) v.51, No.10, p.3030-3034)* in 15 ml abs. DMSO. The reaction was kept for 8 hr at 20°C, then 1.2 ml of 2 M AcOH in DMSO was added and the solution evaporated to dryness in vacuum. The residual oil solidified after water treatment, the precipitate was filtered off, washed with cold water, recrystallized twice from water and dried in vacuum over P_2O_5/KOH to give 1.3 g (yield 67 %) of VII. NMR (Jeol-400, DMSO-d_6) : 4.86 (m, C_1-H), 3.97 (q, CH_3-CH_2-O-), 3.94 (t, $=NO-CH_2-$), 3.79-3.33 (mm, C_3-H, C_6-H, C_5-H, C_2-H, C_4-H), 2.79 (m, $-CH_2-CH_2-S-$), 1.87 (s, CH_3-), 1.23 (t, CH_3-CH_2-O-).

Example VIII

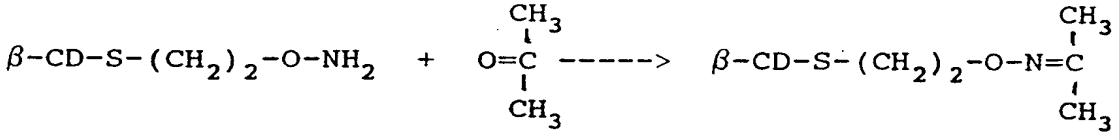
Mono-6-(2-aminooxyethyl)thio-6-deoxy-β-cyclodextrin hydrochloride (VIII)



1.25 g (1.0 mmoles) of (VII) were suspended in 20 ml of 1 N HCl, heating to 50°C gave clear solution, which was evaporated to dryness in vacuum. The residual oil solidified upon treatment with abs. i-PrOH. The precipitate was filtered off, washed with abs. i-PrOH and dried over P_2O_5/KOH in vacuum that gave 0.80 g (65% yield) of (VIII). The amount of aminooxy groups determined as above was 0.88 mmoles/g. NMR (Jeol-400, DMSO-d_6) :4.85 (m, C_1-H), 4.12 (t, H_2NO-CH_2-), 3.82-3.33 (mm, C_3-H, C_6-HC_5-H-C_2-H,C_4-H), 2.86 (m, CH_2-CH_2-S)

Example IX

Acetonoxime of mono-6-(2-aminooxyethyl)thio-6-deoxy-β-cyclodextrin (IX).

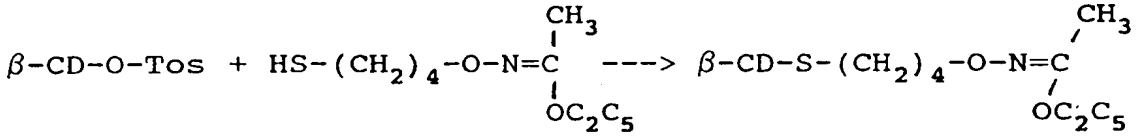


To a solution of 0.62 g (0.5 mmoles) of (VIII) in 8.0 ml of a H_2O-acetone cocktail (1:1, V/V), a diluted water NH_3 solution was added to pH 5-6 and the reaction mixture was incubated for 2 h at 20°C. After evaporation to dryness,

the residue was crystallized twice from water. The precipitate was filtered off, dried in vacuum over P_2O_5/KOH and 0.5 g (80 % yield) of IX as obtained. NMR (Jeol-400, DMSO-d_6) : 4.85 (m, C_1-H), 4.04 (t, =NO-CH_2-), 3.79-3.36 (mm, C_3-H, C_6-H, C_5-H, C_2-H, C_4-H), 2.77 (m, CH_2-CH_2-S), 1.80 (d, CH_3-).

Example X

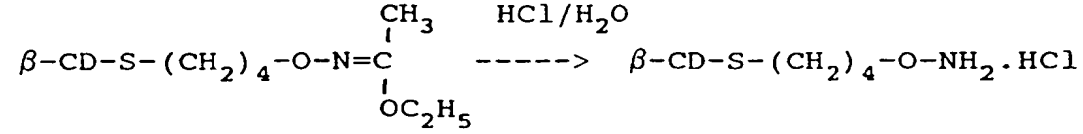
Mono-6-(4-ethoxyethylideneaminooxybutyl)thio-6-deoxy-β-cyclodextrin (X)



Mono-6-(4-ethoxyethylideneaminooxybutyl)thio-6-deoxy-β-cyclodextrin was obtained as is described for (VII) starting from 1.95 g (1.5 mmoles) of mono-6-O-tosyl--β-cyclodextrin and 0.85 g (4.5 mmoles) of 4-ethoxyethylideneaminooxyethylmercaptan *(*Nedospasov *A.A. and Khomutov R.M. (1976) Izv. AN SSSR Ser. Khim. (in Russian) No.9, p.2113-2115)* that gave 1.56 g (80 % yield) of (X). NMR (Jeol-400, DMSO-d_6) : 4.84 (m, C_1-H), 3.95 (q, CH_3-CH_2-O--), 3.80 (t, =NO-CH_2-), 3.75-3.31 (mm, C_3-H, C_6-H, C_5-H, C_2-H, C_4-H), 2.68 (m, CH_2-CH_2-S) 1.87 (d, CH_3-), 1.56 (m, -CH_2-CH_2), 1.23 (t, CH_3-CH_2-O-).

Example XI

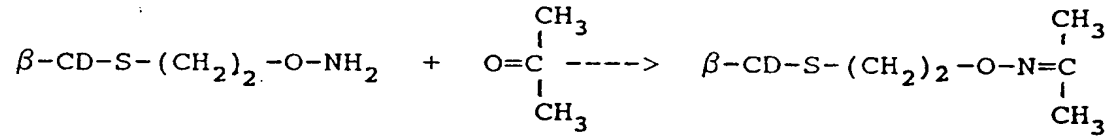
Mono-6-(4-aminooxybutyl)thio-6-deoxy-β-cyclodextrin hydrochloride (XI)



Mono-6-(4-aminooxybutyl)thio-6-deoxy-β-cyclodextrin hydrochloride was obtained as is described for (VIII), starting from 1.3 g (1.0 mmoles) of (X) that gave 1.0 g (75 % yield) of (XI). NMR (Jeol-400, DMSO-d_6) : 10.85 (m, H_2NO-), 4.83 (m, C_1-H), 3.99 (m, H_2NO-CH_2-), 3.63-3.31 (mm, C_3-H, C_6-H, C_5-H, C_2-H, C_4-H), 1.59 (m, $-CH_2-CH_2-$).

Example XII

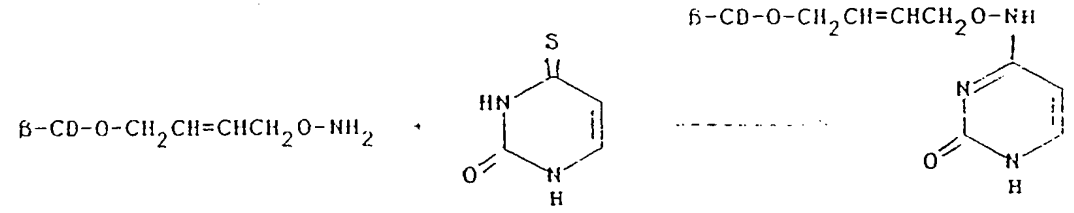
Acetonoxime of mono-6-(2-aminooxyethyl)thio-6-deoxy-β-cyclodextrin (XII).



The acetonoxime of mono-6-(2-aminooxyethyl)thio-6-deoxy-β-cyclo-dextrin was obtained as is described for (IX) starting from 0.7 g of (XI) that gave 0.55 g (78 % yield) of (XII). NMR (Jeol-400, DMSO-d_6) :4.82 (m, C_1-H), 3.89 (t, $=NO-CH_2$-), 3.61-3.32 (mm, C_3-H, C_6-H, C_5-H, C_2-H, C_4-H), 2.92 (m, CH_2-CH_2-S), 1.78 (d, CH_3-), 1.52 (m, $-CH_2-CH_2-$).

Example XIII

Reaction of I with 4-thiouracil

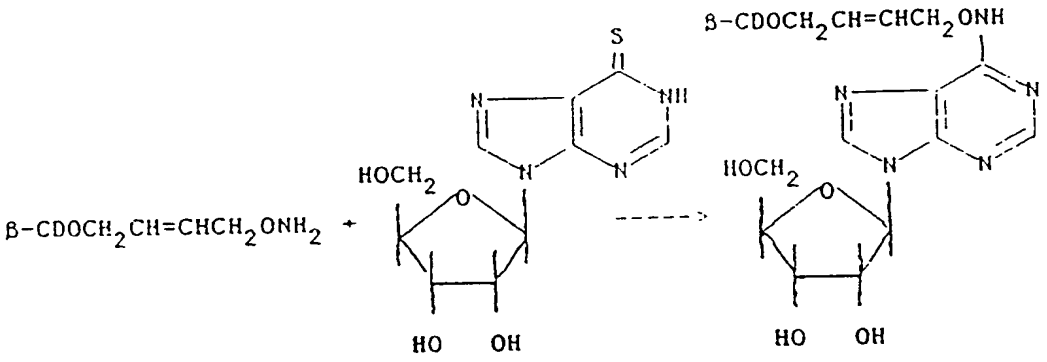


A 1 mM solution of 4-thiouracil (Lachema, Brno, Czechoslovakia) was incubated at 20°C within a 0.1 M solution of 4-aminooxy-2-butenyl-β-CD (I) at neutral pH. The UV-spectra were recorded at certain time intervals using

cuvettes of 1 mm optical path length (Figure 1a). This reaction was compared with the reaction of 4-thiouracil with 1-aminooxybutane under the same reaction conditions (Fig.1b). The results show significantly higher velocity of reaction with the aminooxy-2-butenyl-β-CD.

Example XIV

Reaction of I with 6-mercaptopurine riboside



A 1 mM solution of 6-mercaptopurine riboside (Sigma Chem.Co., USA) was incubated at 20°C within 0.1 M of 4-aminooxy-2-butenyl-β-CD (I). At certain time intervals UV-spectra were recorded in cuvettes of 1 mm optical path (Fig. 2a). The similar reaction with 1-aminooxybutane (Fig. 2b) showed drastically higher reaction rates with the CD-derivative.

WHAT IS CLAIMED IS:

1. Aminooxy-cyclodextrin derivatives of the formula 1:

$$CD - (X - Y - ONH_2)_n \quad , \qquad (1)$$

wherein
CD is a mono- or polydeoxy α-, β- or γ-cyclodextrin, carrying in its 6-, 3- and/or 2-position the aminooxy function containing group ($X-Y-ONH_2$), and optionally carrying further substituents different from ($X-Y-ONH_2$) in their 6-, 3- and/or 2-positions, and wherein Y is a linker group between the aminooxy group and the mono- or polydeoxy-CD-group,
X is a functional group or an atom necessary to connect the linker Y and the deoxy CD group, or Y is a direct bond when X is a direct bond,
and n is ≥ 1, but ≤ 24, 21 and 18 for α-, β- or γ-cyclodextrin, respectively, as well as the aminooxy protected derivatives thereof, especially ethoxy-ethylidene protected aminooxy and acetone oxime derivatives thereof.

2. A derivative according to claim 1, wherein Y and X are both direct bonds.

3. A derivative according to claim 1 or 2, wherein one or more of the primary hydroxy groups at a 6-position of α-, β- or γ-CD are substituted with a $X-Y-ONH_2$ fragment, wherein X and Y have the meaning of claim 1.

4. A derivative according to any one of claims 1 and 3, wherein Y is a linear or branched alkylene, alkenylene with one or more double bounds which may be either isolated or conjugated, alkynylene with one or more triple bonds which may be either isolated or conjugated, or arylene or arylalkylene fragments where aryl may be substituted or not substituted, whereby the alkylene, alkenylene and alkynyle-

ne fragments may be linear or branched and preferably contain 2-12 C-atoms in the chain, and one or more of the chain members (methylene groups) may be replaced by -NH-, -O-, -S-, -S-S-, -C(O)NH, -C(O)O-, -OP(O)(OH)O-, -S(O)-, SO_2-, -CHR-, where R is preferably alkyl, aryl, -OR', $-NH_2$, -NHR', $-NR'_2$, -OH, -COOH, or $-ONH_2$ groups and where R' is alkyl, aryl, or acyl.

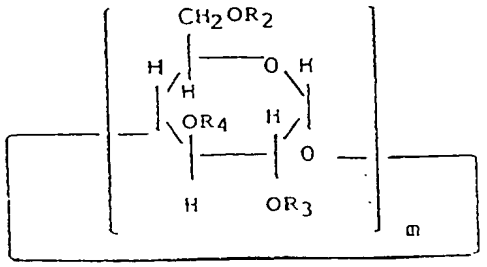
5. A derivative according to any one of the claims 1, 3 and 4, wherein X is -O-, -S-, -NH-, -NR"-, -OCO-, -NH-O-, =NO-, -NHC(O)-, -OP(O)(OH), -R"C=NO-, where R" is linear or branched lower alkyl.

6. A derivative according to the claims 4 or 5, wherein Y is alkylene containing 2-12 C-atoms, wherein one or more of the chain members may be replaced by -NH-, -O-, -S-, -C(O)NH-, -C(O)O-, or CHR_1 wherein R_1 is methyl, ethyl or propyl and X is -O-, -S-, -NH-, -OC(O)-, and -NH-C(O)-.

7. Any compound according to claim 1-6, wherein one or more of the hydroxyl groups at 6-, 3-, and/or 2- position(s) are substituted with a group, for example, H_2N- , HS-, -COOH, alkoxy- , such as C_1 - C_6- alkoxy- , aryloxy-, wherein aryl is preferably phenyl, benzyl, or tolyl, or with acyloxy group, wherein acyl preferably originates from C_1 - C_6- carboxyl, or benzoic acids, and wherein alkyl-, aryl-, and acyloxy- can additionally contain functional groups like H_2N- , HS-, -COOH in their structure, in side chain or in aromatic ring.

8. Method according to claim 1 or 3 for preparing compound of formula 1, wherein X is O, and wherein:

a) cyclodextrin of formula (3)



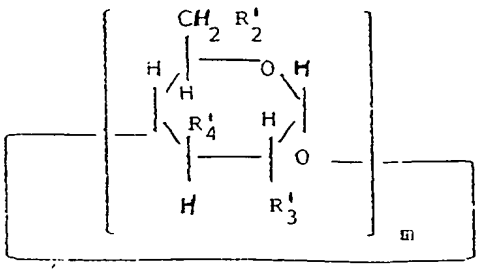
(3)

including R_2, R_3, and R_4 are hydroxyl groups or substituents defined in claim 7, exemplified by unsubstituted alkoxy, like C_1 - C_6- alkoxy or aryloxy like phenyl-, benzyl-, tolyl-, or acyloxy, in which substituents' functional groups, if they exist, are protected whenever necessary, whereby at least one of the positions 6, 3, and/or 2 contain hydroxyl group, preferably 6- hydroxy group, is alkylated with a compound according to formula (3'):

$$Z - Y - ON = C(CH_3) W \qquad (3')$$

wherein W means group $-OC_2H_5$ or $-CH_3$, m and Y are as defined in claims 1 or 3, and Z is a reactive group, preferably Cl, Br, I, tosyl, mesyl or epoxy group, and optionally protecting group(s) is/are removed, or

b) a cyclodextrin derivative of formula (4) is alkylated



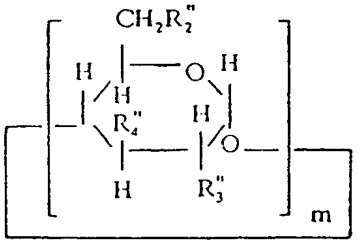
(4)

wherein R'_2, R'_3, R'_4 are hydroxy or activated groups like tosyl, mesyl, halogen, ester, epoxy, preferably tosyl or halogen, possibly bound through a linker group, like alkylen, or substituent as defined in claim 7, said substituent being in a protected form if necessary, whereby the CD-derivative contains at least one of said activated groups with the compound of formula (4')

$$HX - Y- ON = C (CH_3) W \qquad (4')$$

wherein X and Y are as in claim 1, or as in 3 and 4, and X is preferably S or HN- fragment and Y has the meaning defined in claim 6, and W is defined as above, and protecting group(s) is/are possibly removed if necessary, or

c) a cyclodextrin derivative of compound with formula (5)



(5)

wherein at least one of the groups R''_2, R''_3, and R''_4 mean thiol-, amino-, karboxy- etc. group possibly linked directly to deoxy-CD-ring, or mean alkylenoxy- or acyloxy groups, which contain at least one thiol-, amino-, karboxy-, etc. group, or their derivative, and the remaining funtional groups are hydroxyl groups or they have the meaning described in claim 7 for the substituents, and exist, if necessary, in a protected form, typical example being unsubstituted alkoxy, aryloxy, or acyloxy, modified with an appropriate aminooxy protected substituted hydroxylamine according to formula (3'), after which the protecting

group(s) are removed, or

d) CD-derivative of formula (5), which contains one or more of keto or aldehyde groups, possibly bound through a linker group, is allowed to react with bisaminooxy alkanes of formula (5')

$H_2NO\ (CH_2)_tONH_2$ (5')

wherein t is 2-12, and wherein one of the methylene groups can be substituted with oxygen or sulfur atom, or wherein -NH- or -S-S- groups, and a protecting group is removed if necessary.

9. The use of any of the CD-derivatives of claims 1-7 for preparation of oximes with ketones or aldehydes, for preparation of aminooxy derivatives of nucleotide- and nucleoside pyrimidines or purines, or for preparation of inclusion complexes with guest molecules by said CD-derivatives.

10. Oximes of any one of the aminooxy-CDs of claim 1-7 with a synthetic or natural aldehydes or ketones.

11. Derivatives of nucleotide or nucleoside pyrimidines or purines with aminooxy-CDs, wherein aminooxy group is linked to heterocyclic ring, preferably through pyrimidine C-4 and purine C-6, and wherein pyrimidine and purine are preferably cytosine or adenine as such or as their derivatives.

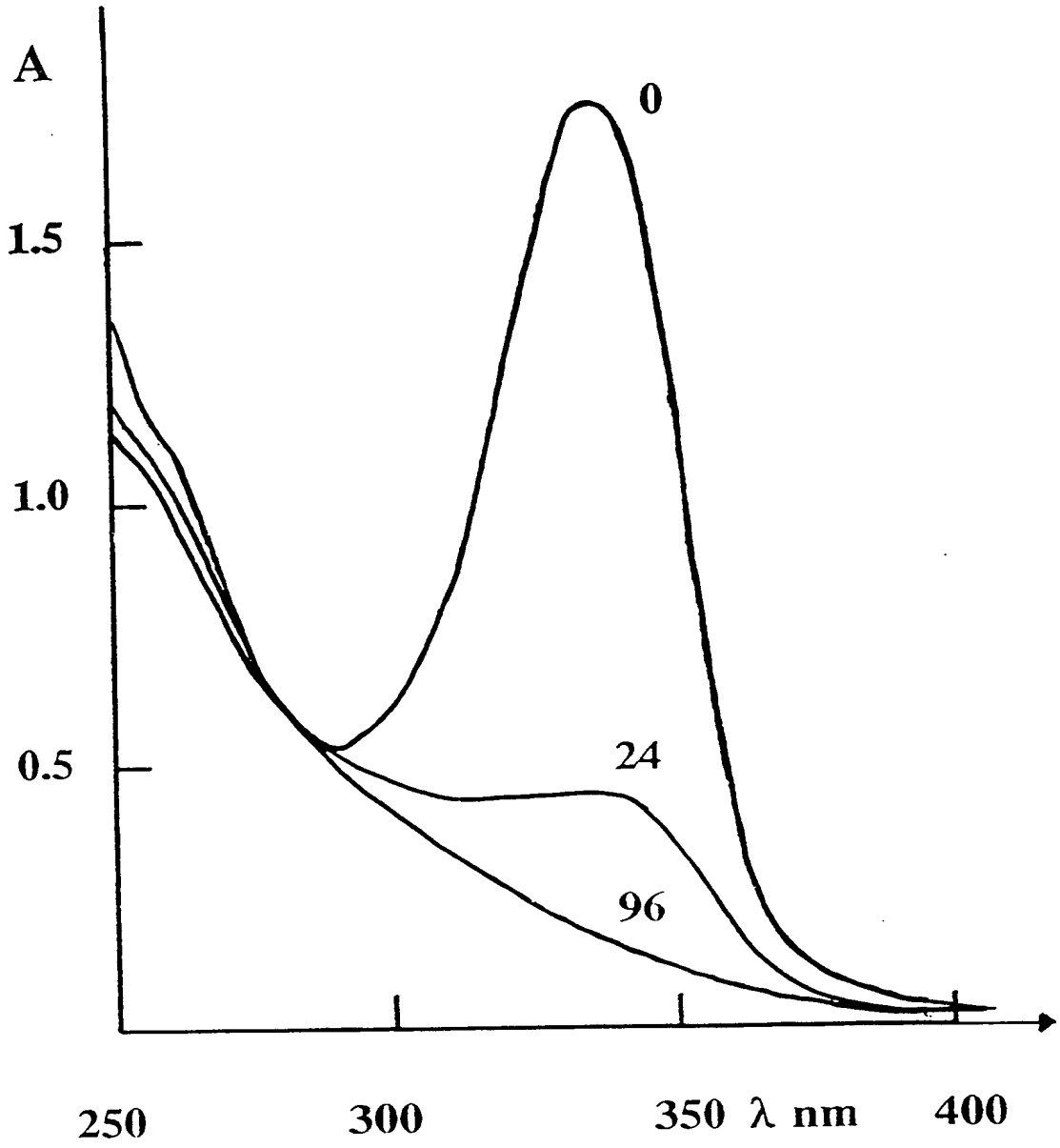


Figure 1 a

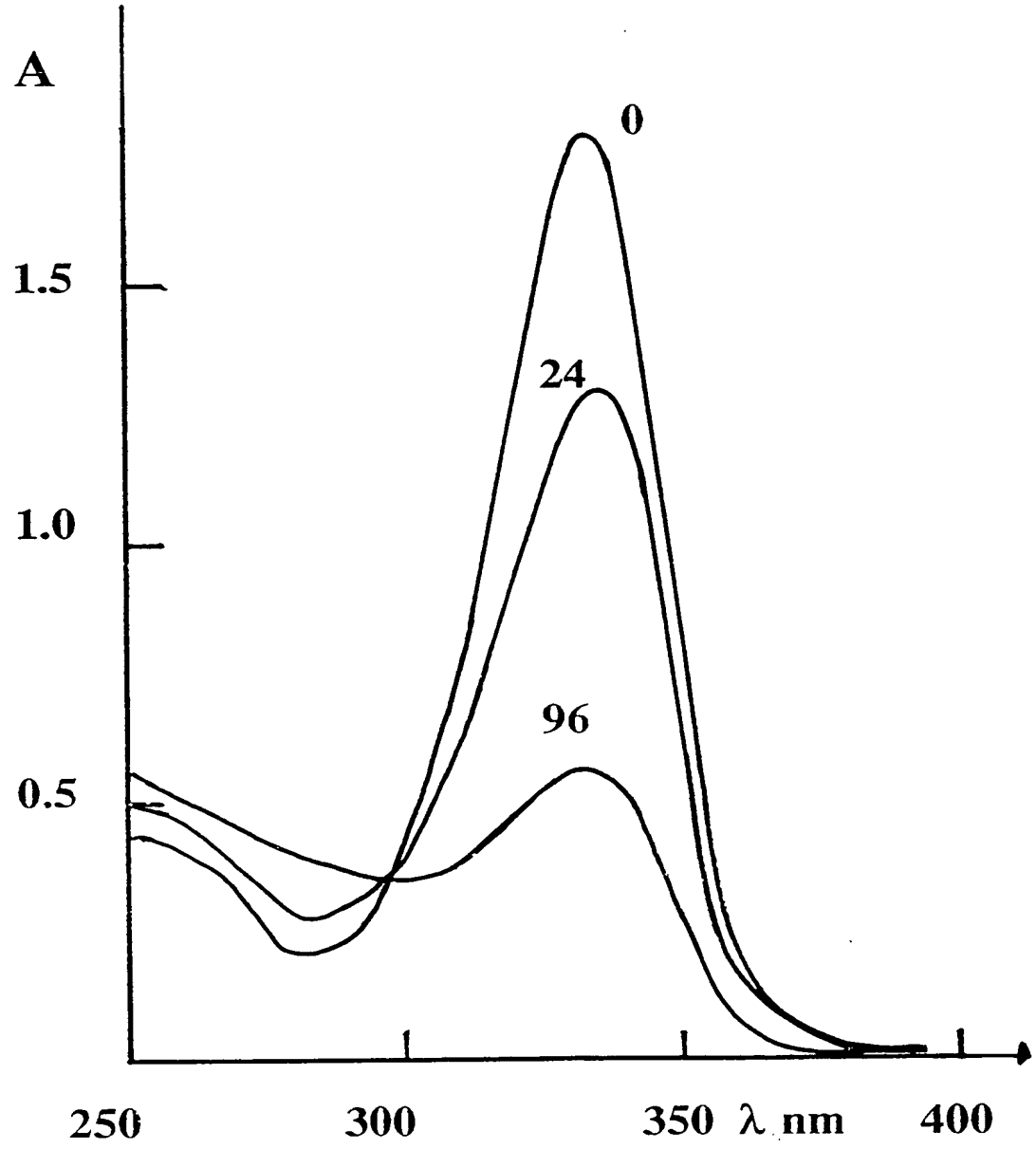


Figure 1 b

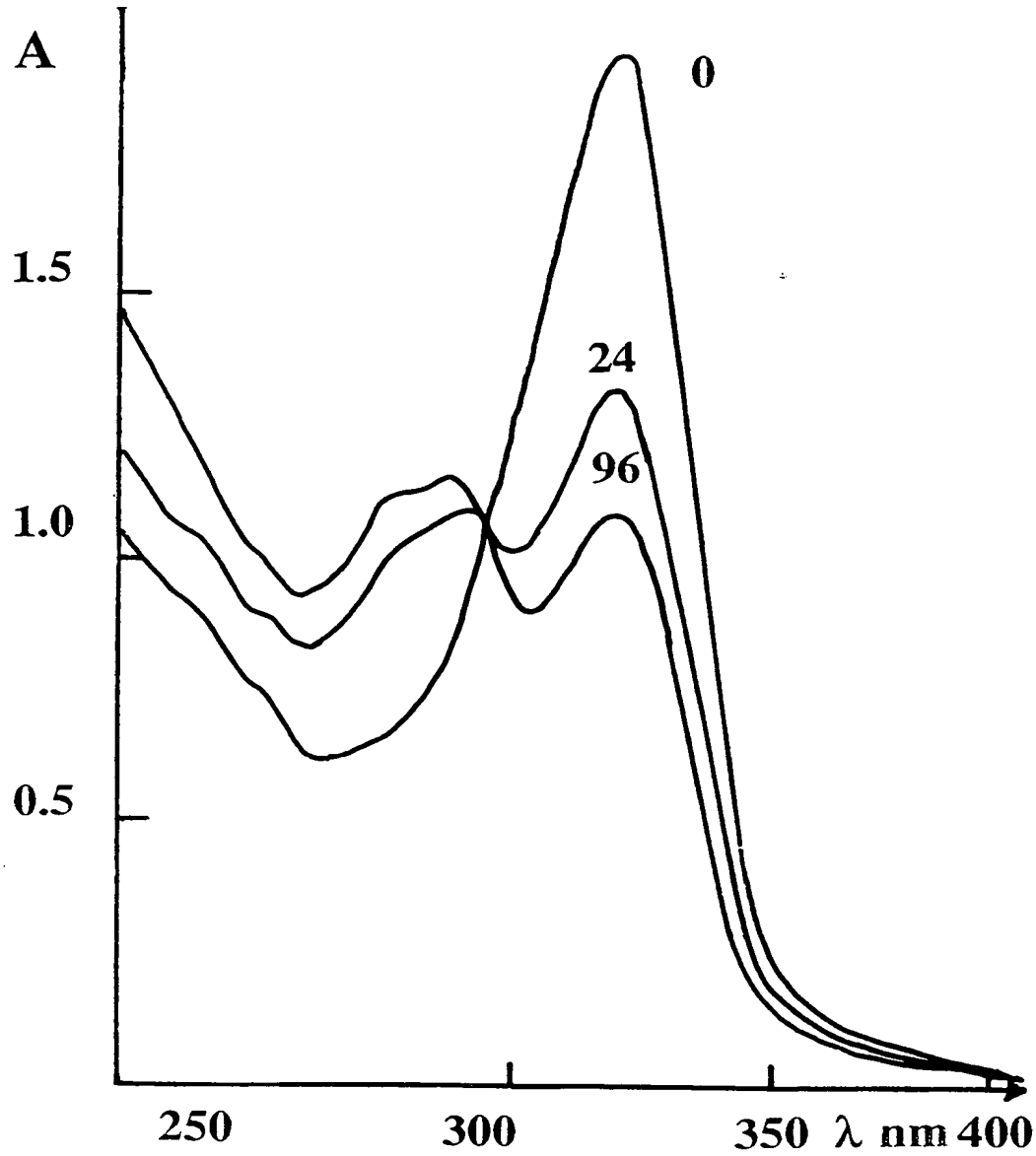


Figure 2 a

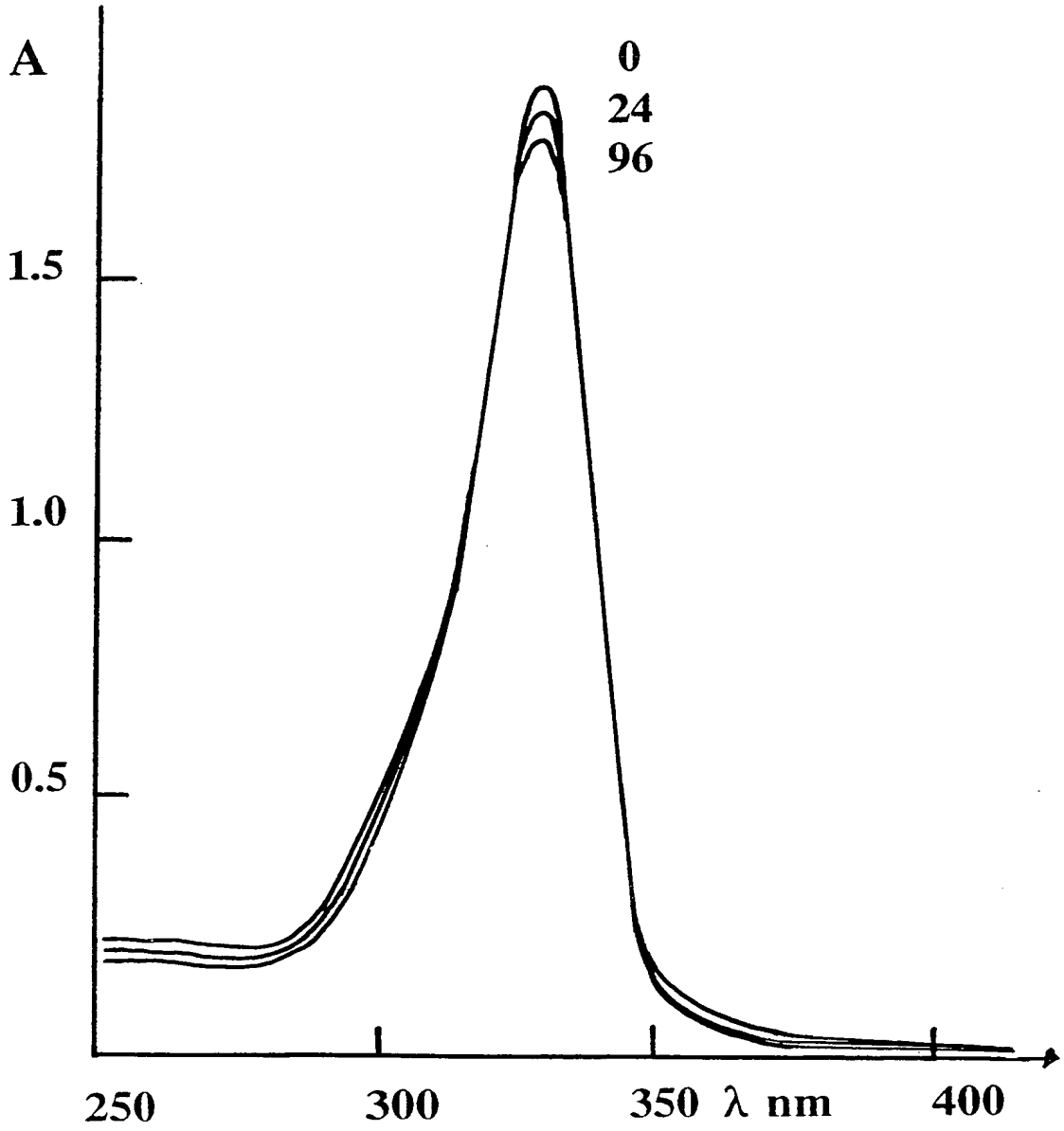


Figure 2 b

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 99/00167

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C08B 37/16, A61K 47/40
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C08B, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, REGISTRY, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9641813 A2 (OFFORD, ROBIN), 27 December 1996 (27.12.96), page 10, 3rd paragraph - page 11, 1st paragraph; page 13, 1st paragraph; page 20, 2nd paragraph --	1-11
X	STN International, File CAPLUS, CAPLUS accession no. 1976:526050, document no. 85:126050, Nedospasov, A. A. et al: "Synthesis and some properties of aminooxyalkyl celluloses"; & Izv. Akad. Nauk SSSR, Ser. Khim. (1976), (5), 1136-41 --	1-9

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 June 1999	23-06-1999
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Helena Danielsson/EÖ Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 99/00167

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Bioorganic Chemistry, Volume 14, 1986, D. V. P. R. Varaprasad et al, "Synthesis of Polyfunctional Hydroxamic Acids for Potential Use in Iron Chelation Therapy" page 8 - page 16	1-9
Y	STN International, File CAPLUS, CAPLUS accession no.1978:510174, document no. 89:110174, Nedospasov, A. A. et al: "Synthesis of aminooxydextrans and adsorbents based on them"; & Izv, Akad. Nauk SSSR, Ser. Khim. (1978), (4), 962-4	1-9
A	File WPI, Derwent accession no. 88-153418, AS USSR MOLECULAR et al: "Prepn. of amino hydroxybutenyl cellulose - by alkenylating cellulose with excess of ethoxy-ethylidene-amino-hydroxy bromobutene in diluted aq. sodium hydroxide"; & SU,A,1348345, 871030	1-7
A	Bioorganic & Medicinal Chemistry Letters, Volume 4, No 16, 1994, Mark A. Mortellaro et al, "Synthesis of beta-Cyclodextrin Oximes" page 2041 - page 2044	1-10
P,X	STN International, File CAPLUS, CAPLUS accession no. 1998:600670, document no. 129:302779, Hori, Yuji et al: "Effects of solvents on the chirality of ferrichrome-mimicking Fe3+ complexes based on .alpha.-cyclodextrin"; & Nippon Kagaku Kaishi (1998 September), 602-608	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/06/99

International application No.
PCT/FI 99/00167

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9641813 A2	27/12/96	AU 7327296 A	09/01/97
		CA 2204726 A	27/12/96
		EP 0788375 A	13/08/97
		JP 10509208 T	08/09/98

PATENT COOPERATION TREATY

PCT

REC'D 21 JUN 2000
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference CD-derivative	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/FI99/00167	International filing date *(day/month/year)* 04.03.1999	Priority date *(day/month/year)* 04.03.1998
International Patent Classification (IPC) or national classification and IPC7 C08B 37/16, A61K 47/40		
Applicant KHOMUTOV, Alexei Radievich et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02.09.1999	08.06.2000
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. 08-667 72 88	Authorized officer Helena Danielsson/MP Telephone No. 08-782 25 00

Telex 17978 PATOREG-S

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/FI99/00167

I. Basis of the report

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

☒ the international application as originally filed.

☐ the description, pages _____ , as originally filed,
pages _____ , filed with the demand,
pages _____ , filed with the letter of _____ ,
pages _____ , filed with the letter of _____ .

☐ the claims, Nos. _____ , as originally filed,
Nos. _____ , as amended under Article 19,
Nos. _____ , filed with the demand,
Nos. _____ , filed with the letter of _____ ,
Nos. _____ , filed with the letter of _____ .

☐ the drawings, ~~sheets~~/fig _____ , as originally filed,
~~sheets~~/fig _____ , filed with the demand
~~sheets~~/fig _____ , filed with the letter of _____ ,
~~sheets~~/fig _____ , filed with the letter of _____ .

2. The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, ~~sheets~~/fig _____

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/FI99/00167

V. Res ned statement under Article 35(2) with regard t novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-11	YES
	Claims		NO
Inventive step (IS)	Claims	1-11	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-11	YES
	Claims		NO

2. Citations and explanations

The claimed invention relates to the design and synthesis of chemical derivatives of cyclodextrins.

The invention intends to enlarge the area of application of cyclodextrins.

This is accomplished, according to the invention, by the preparation of an aminooxy-cyclodextrin, which have significantly different properties compared with cyclodextrin derivatives known in the prior art.

The documents cited in the International Search reports were:

D1 WO 96 41813 A1
D2 CA Abstract 1998:600670
D3 CA Abstract, accession no. 1978:510174
D4 Bioorganic & Medicinal Chemistry Letters, vol. 4, no. 16,pp. 2041-2044, 1994

Document D1 discloses polymers, such as dextrin, which are aminooxy-functionalised. This document also discloses the preparation of the aminooxy-functionalised polymer. The functionalised dextrin can be used for site-specific modification of for example polypeptides. The invention differs from D1 in that D1 does not mention cyclodextrin. It is not considered obvious for a person skilled in the art with the knowledge of D1 to prepare an aminooxy-cyclodextrin derivative or an aminooxy protected derivative thereof, because the hydroxy groups of cyclodextrin is not reacting in the same way as the hydroxy groups in linear dextrin. Thus it is not possible to predict the outcome of a reaction with cyclodextrin based on a similar reaction with linear dextrin.

.../...

Supplemental B x
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V.

In D2 an aminooxybutyl cellulose prepared by treating a cellulose with (α-ethoxy-ethylidene-aminooxy) butane is disclosed and then removing the protecting group. The difference between the claimed invention and D2 is that cyclodextrin is not mentioned in D2.

In D3 an aminooxybutyl dextran obtained by alkylation of a sodium salt of dextran is disclosed. Aminooxybutyl sephadex was obtained analogously. Both modified dextrans were useful as adsorbents. The difference between the invention claimed in claims 1-8 and this document is that this document does not mention cyclodextrin.

It is not considered that the chemistry of cellulose and dextran resembles the chemistry of cyclodextrin enough for making it obvious for the person skilled in the art with the knowledge of D2 or D3 to prepare an aminooxy-cyclodextrin derivative as well as an aminooxy protected derivative thereof.

Further, D4 makes known a β-cyclodextrin oxime. However, this oxime is prepared by air oxidation of cyclodextrin hydroxylamine, and not aminooxy cyclodextrin and the claimed invention does therefore differ from D4.

In view of the above, it is considered that the invention claimed in claims 1-11 fulfils the requirements of novelty, technical applicability and inventive step.

Copy for the Elected Office (EO/US) PCT/FI99/00167

ATENT COOPERATION TR .TY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

OY JALO ANT-WUORINEN AB
Iso Roobertinkatu 4-6 A
FIN-120 Helsinki
FINLANDE

Date of mailing (day/month/year)
15 June 2000 (15.06.00)

Applicant's or agent's file reference
CD-derivative

IMPORTANT NOTIFICATION

International application No.
PCT/FI99/00167

International filing date (day/month/year)
04 March 1999 (04.03.99)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [ ] the agent [X] the common representative

Name and Address
KORPELA, Timo, Kalevi
Kasarminkatu 5 as 8
FIN-20500 Turku
Finland

State of Nationality:
State of Residence:
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Facsimile No.: +358 2 333 80 80
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
OY JALO ANT-WUORINEN AB
Iso Roobertinkatu 4-6 A
FIN-120 Helsinki
Finland

State of Nationality:
State of Residence:
Telephone No.: 358 9 612 6120
Facsimile No.: 358 9 640 575
Teleprinter No.:

3. Further observations, if necessary:
Please note that OY JALO ANT-WUORINEN AB has been appointed as agent of record.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
A. Karkachi
Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 03 November 1999 (03.11.99)	
International application No. PCT/FI99/00167	**Applicant's or agent's file reference** CD-derivative
International filing date (day/month/year) 04 March 1999 (04.03.99)	**Priority date** (day/month/year) 04 March 1998 (04.03.98)
Applicant KHOMUTOV, Alexei Radievich et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

02 September 1999 (02.09.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer J. Leitao
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

KORPELA, Timo, Kalevi
Kasarminkatu 5 as 8
FIN-20500 Turku
FINLANDE

Date of mailing (day/month/year)
03 November 1999 (03.11.99)

Applicant's or agent's file reference
CD-derivative

IMPORTANT NOTIFICATION

International application No.
PCT/FI99/00167

International filing date (day/month/year)
04 March 1999 (04.03.99)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address
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Profsoyusnaya Street 114, Corp. 6
Moskow, 117437
Russian Federation

State of Nationality: RU
State of Residence: US
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
YAKOVLEV, Dmitry Yurievich
Apartment 46
Profsoyusnaya Street 114, Corp. 6
Moskow, 117420
Russian Federation

State of Nationality:
State of Residence:
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
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Authorized officer
J. Leitao
Telephone No.: (41-22) 338.83.38